2-14-00

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	**L0461/7081**
First Named Inventor or Application Identifier	**Scanlan et al.**
Express Mail Label No.	**EL024661754US**
Date of Deposit	**February 11, 2000**

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

1. X Fee Transmittal Form
   (Submit an original, and a duplicate for fee processing)
2. X Specification [Total pages 29]
   16 pages description
   1 page abstract
   12 pages claims
   56 claims
3. Drawing(s) *(35 USC 113)* [Total sheets ___]
   ☐ Informal ☐☐Formal [Total drawings ___]
4. X Oath or Declaration [Total pages 3]
   a. ☐ Newly executed (original or copy)
   b. X Copy from a prior application (37 CFR 1.63(d))
   (for continuation/divisional with Box 17 completed)
   [Note Box 5 below]
   i. ☐ DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
5. X Incorporation by Reference
   (usable if Box 4b is checked)
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Microfiche Computer Program (Appendix)
7. X Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
   a. X Computer Readable Copy **(see "other" below)**
   b. X Paper Copy (identical to computer copy)
   c. X Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & documents(s))
9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney
   (when there is an assignee)
10. ☐ English Translation of Document *(if applicable)*
11. X Information Disclosure ☐ Copies of IDS
    Statement (IDS)/PTO-1449 Citations
12. X Preliminary Amendment
13. X Return Receipt Postcard (MPEP 503)
    (Should be specifically itemized)
14. ☐ Small Entity ☐ Statement filed in prior
    Statement(s) application, Status still proper and desired
15. ☐ Certified Copy of Priority Document(s)
    (if foreign priority is claimed)

16. **Other:** Associate Power of Attorney; Letter requesting use of previous computer readable form; copy of computer disk label from previous case.

17. **If a CONTINUING APPLICATION,** check appropriate box and supply the requisite information:

☐ Continuation X Divisional ☐ Continuation-in-part (CIP) of prior application No.: 08/948,705

X Cancel in this application original claims 1-5, 7-36 and 41-56 of the prior application before calculating the filing fee.

X Amend the specification by inserting before the first line the sentence:

This application is a divisional of application serial no. 08/948,705, filed October 10, 1997, entitled ISOLATED NUCLEIC ACID MOLECULES ASSOCIATED WITH COLON CANCER AND METHODS FOR DIAGNOSING AND TREATING COLON CANCER, and now pending.

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DATE	FEBRUARY 11, 2000

Express Mail Label No:
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Attorney's Docket No. **L0461/7081**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Scanlan et al.
Serial No: Unknown
Filed: Herewith
For: ISOLATED NUCLEIC ACID MOLECULES ASSOCIATED WITH COLON CANCER AND METHODS FOR DIAGNOSING AND TREATING COLON CANCER
Examiner: Not Assigned
Art Unit: Unknown

Box Patent Application
ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please enter this Preliminary Amendment as follows:

In the Specification

On page 4, line 12, please delete "Applications Serial".

On page 4, lines 13-14, please delete "08/580,980 , and Application Serial No. 08/479,328, filed on June 7, 1995 and January 3, 1996, respectively" and insert --5,698,396-- therefor.

On page 4, line 14, please delete "All three" and insert --Both-- therefor.

On page 6, line 5, please delete "with the IgG then interacting".

On page 6, line 19, please delete "allowed U.S. Patent Application Serial No. 08/479,328" and insert --U.S. Patent 5,698,396-- therefor.

On page 9, line 18, please delete "300-400" and insert --300 to 400-- therefor.

On page 10, line 1, please delete "screening" and insert --screened-- therefor.

On page 10, line 20, please delete "breain" and insert --brain-- therefor.

On page 11, line 2, please delete "wherein," and insert --, wherein-- therefor.

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In the Claims

Please cancel without prejudice claims 1-5, 7-36 and 41-56.

Please amend the claims as follows:

6.(amended) An [I]isolated protein encoded by [the] an isolated nucleic acid [of claim 1] molecule selected from the group consisting of:

(a) nucleic acid molecules which encode a cancer associated antigen, and which comprise a nucleotide sequence, the complementary sequence of which hybridizes, under stringent conditions, to at least one second nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the nucleotide sequences set forth as SEQ ID NOs: 1, 2, 3, 4, and 5,

(b) nucleic acid molecules that differ from the nucleic acid molecules of (a) in codon sequence due to the degeneracy of the genetic code, and

(c) complements of (a) or (b).

37.(amended) A composition of matter useful in stimulating an immune response to at least one protein encoded by at least one nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5, said composition comprising a plurality of peptides derived from the amino acid sequence of said at least one protein, wherein said peptides bind to one or more MHC molecules presented on the surface of cells which express an abnormal amount of said at least one protein.

Please add the following new claims:

57. The isolated protein of claim 6, wherein the nucleic acid molecule comprises SEQ ID NO:1.

58. The isolated protein of claim 6, wherein the nucleic acid molecule comprises SEQ ID NO:2.

59. The isolated protein of claim 6, wherein the nucleic acid molecule comprises SEQ ID NO:3.

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60. The isolated protein of claim 6, wherein the nucleic acid molecule comprises SEQ ID NO:4.

61. The isolated protein of claim 6, wherein the nucleic acid molecule comprises SEQ ID NO:5.

62. The composition of matter of claim 37, wherein the at least one nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:1.

63. The composition of matter of claim 37, wherein the at least one nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:2.

64. The composition of matter of claim 37, wherein the at least one nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:3.

65. The composition of matter of claim 37, wherein the at least one nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:4.

66. The composition of matter of claim 37, wherein the at least one nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:5.

67. The composition of matter of claim 37, wherein at least one of the plurality of peptides is coupled to an immune response stimulating compound.

REMARKS

Please enter this Preliminary Amendment before calculating the fees. Applicants have deleted all claims but those corresponding to claim group number 2 (claims 6 and 37-40) as set forth in the restriction requirement mailed on October 1, 1998 in the parent case, serial number 08/948,705. Amendments to the Specification are the same as made in the parent application.

New claims 57-61 and 62-66 are derived from original claims 6 and 37, respectively. Support for new claim 67 can be found in the Specification at page 14, line 16. No new matter has been added.

Respectfully submitted,

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ATTORNEY DOCKET NO. L0461/7081
Date: February 11, 2000
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ISOLATED NUCLEIC ACID MOLECULES ASSOCIATED WITH COLON CANCER AND METHODS FOR DIAGNOSING AND TREATING COLON CANCER

FIELD OF THE INVENTION

This invention relates to the isolation of genes associated with colon cancer, methods of diagnosing colon cancer using these, as well as other genes which are known, as well as therapeutic approaches to treating such conditions.

BACKGROUND AND PRIOR ART

It is fairly well established that many pathological conditions, such as infections, cancer, autoimmune disorders, etc., are characterized by the inappropriate expression of certain molecules. These molecules thus serve as "markers" for a particular pathological or abnormal condition. Apart from their use as diagnostic "targets", i.e., materials to be identified to diagnose these abnormal conditions, the molecules serve as reagents which can be used to generate diagnostic and/or therapeutic agents. A by no means limiting example of this is the use of cancer markers to produce antibodies specific to a particular marker. Yet another non-limiting example is the use of a peptide which complexes with an MHC molecule, to generate cytolytic T cells against abnormal cells.

Preparation of such materials, of course, presupposes a source of the reagents used to generate these. Purification from cells is one laborious, far from sure method of doing so. Another preferred method is the isolation of nucleic acid molecules which encode a particular marker, followed by the use of the isolated encoding molecule to express the desired molecule.

To date, two strategies have been employed for the detection of such antigens, in e.g., human tumors. These will be referred to as the genetic approach and the biochemical

approach. The genetic approach is exemplified by, e.g., dePlaen et al., Proc. Natl. Sci. USA 85: 2275 (1988), incorporated by reference. In this approach, several hundred pools of plasmids of a cDNA library obtained from a tumor are transfected into recipient cells, such as COS cells, or into antigen-negative variants of tumor cell lines. Transfectants are screened for the expression of tumor antigens via their ability to provoke reactions by anti-tumor cytolytic T cell clones. The biochemical approach, exemplified by, e.g., Mandelboim, et al., Nature 369: 69 (1994) incorporated by reference, is based on acidic elution of peptides which have bound to MHC-class I molecules of tumor cells, followed by reversed-phase high performance liquid chromography (HPLC). Antigenic peptides are identified after they bind to empty MHC-class I molecules of mutant cell lines, defective in antigen processing, and induce specific reactions with cytolytic T-lymphocytes ("CTLs"). These reactions include induction of CTL proliferation, TNF release, and lysis of target cells, measurable in an MTT assay, or a ^{51}Cr release assay.

These two approaches to the molecular definition of antigens have the following disadvantages: first, they are enormously cumbersome, time-consuming and expensive; second, they depend on the establishment of CTLs with predefined specificity; and third, their relevance in vivo for the course of the pathology of disease in question has not been proven, as the respective CTLs can be obtained not only from patients with the respective disease, but also from healthy individuals, depending on their T cell repertoire.

The problems inherent to the two known approaches for the identification and molecular definition of antigens is best demonstrated by the fact that both methods have, so far, succeeded in defining only very few new antigens in human tumors. See, e.g., van der

Bruggen et al., Science 254: 1643-1647 (1991); Brichard et al., J. Exp. Med. 178: 489-495 (1993); Coulie, et al., J. Exp. Med. 180: 35-42 (1994); Kawakami, et al., Proc. Natl. Acad. Sci. USA 91: 3515-3519 (1994).

Further, the methodologies described rely on the availability of established, permanent cell lines of the cancer type under consideration. It is very difficult to establish cell lines from certain cancer types, as is shown by, e.g., Oettgen, et al., Immunol. Allerg. Clin. North. Am. 10: 607-637 (1990). It is also known that some epithelial cell type cancers are poorly susceptible to CTLs in vitro, precluding routine analysis. These problems have stimulated the art to develop additional methodologies for identifying cancer associated antigens.

One key methodology is described by Sahin, et al., Proc. Natl. Acad. Sci. USA 92: 11810-11913 (1995), incorporated by reference. Also, see U.S. Patent Applications Serial No. 08/580,980, and Application Serial No. 08/479,328, filed on June 7, 1995 and January 3, 1996, respectively. All three of these references are incorporated by reference. To summarize, the method involves the expression of cDNA libraries in a prokaryotic host. (The libraries are secured from a tumor sample). The expressed libraries are then immunoscreened with absorbed and diluted sera, in order to detect those antigens which elicit high titer humoral responses. This methodology is known as the SEREX method ("Serological identification of antigens by Recombinant Expression Cloning"). The methodology has been employed to confirm expression of previously identified tumor associated antigens, as well as to detect new ones. See the above referenced patent applications and Sahin, et al., supra, as well as Crew, et al., EMBO J 144: 2333-2340

(1995).

The SEREX methodology has now been applied to colon cancer samples. Several nucleic acid molecules have been newly isolated and sequenced, and are now associated with stomach cancer. Further, a pattern of expression involving these, as well as previously isolated genes has been found to be associated with colon cancer. These results are the subject of this application, which is elaborated upon in the disclosure which follows.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Tumor samples were obtained as surgical samples, and were frozen at -80°C until ready for use.

Total RNA was then isolated from the samples, using the well known guanidium thiocyanate method of Chirgwin, et al., Biochemistry 18: 5294-5299 (1979), incorporated by reference. The thus obtained total RNA was then purified to isolate all poly A^+ RNA, using commercially available products designed for this purpose.

The poly A^+ RNA was then converted into cDNA, and ligated into λZAP, a well known expression vector.

Three cDNA libraries were constructed in this way, using colorectal carcinoma samples. A fourth library, also from colorectal carcinoma, was prepared, albeit in a different way. The reasons for this difference will be clear in the examples, infra.

The fourth library was an IgG subtraction library, prepared by using a subtraction partner, generated by PCR amplification of a cDNA clone which encoded an IgG molecule.

See, e.g., Ace et al, Endocrinology 134: 1305-1309 (1994), and incorporated by reference in its entirety.

This is done to eliminate any false, positive signals resulting from interaction of cDNA clones which encode IgG, with the IgG then interacting with the anti-human IgG used in the assay, as described infra. PCR product was biotinylated, and hybridized with denatured second strand cDNA, at 68°C for 18 hours. Biotinylated hybrid molecules were coupled to streptavidin, and then removed by phenol chloroform extraction. Any remaining cDNA was also ligated into λZAP. All libraries were amplified, prior to immunoscreening discussed infra.

Example 2

Immunoscreening was carried out, using sera obtained from patients undergoing routine diagnostic and therapeutic procedures. The sera were stored at -70°C prior to use. Upon thawing, the sera were diluted at 1:10 in Tris buffered saline (pH 7.5), and were then passed through Sepharose 4B columns. First, the sera were passed through columns which had E. coli Y1090 lysates coupled thereto, and then lysates from bacteriophage infected E. coli BNN97 lysates. Final serum dilutions were then prepared in 0.2% non-fat dried milk/Tris buffered saline.

The method of Sahin et al., Proc. Natl. Acad. Sci. USA 92: 11810-11813 (1995), and allowed U.S. patent application Serial No. 08/479,328, both of which are incorporated by reference, was used, with some modifications. Specifically recombinant phage at a concentration of $4x10^3$ phages per 15 cm plate (pfus), were amplified for six hours, after

which they were transferred to nitrocellulose membranes for 15 hours. Then, the membranes were blocked with 5% nonfat dried milk.

As an alternative to the IgG subtraction, discussed supra, membranes were prescreened in a 1:2000 dilution of peroxidase conjugated, Fc fragment specific goat anti-human IgG, for one hour, at room temperature. Color was developed using 3,3-diaminobenzidine tetrahydrochloride, which permitted scoring of IgG encoding clones.

Membranes were then incubated in 1:100 dilutions of autologous sera, which had been pretreated with the Sepharose 4B columns, as described supra. The filters were then incubated, in a 1:3000 dilution of alkaline phosphatase conjugated Fc fragment specific, goat anti-human IgG, for one hour, at room temperature. The indicator system 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate was then added, and color development assessed. Any positive clones were subcloned, and retested, except the tine on the nitrocellulose membrane was reduced to three hours. A total of forty-eight positive clones were identified.

Analysis of probes for SEQ ID NOS: 1 and 2 confirmed their universal expression.

Example 3

Example 2 described work using autologous serum. The positive clones were then rescreened, using allogeneic serum, following the same method discussed supra, in example 2, except IgG prescreening was omitted. The allogeneic sera was obtained from sixteen normal blood donors, and twenty nine patients who had been diagnosed with colorectal cancer.

The analysis with the two types of serum revealed that fourteen reacted with a subset of sera from normal and cancer patients, twenty-eight only with autologous sera, and six with both allogeneic and autologous sera. Over 60% of the allogeneic serum samples tested reacted with at least one of these positive clones. About 20% reacted with two or more.

Example 4

In view of the results described in example 3, further experiments were carried out using serum samples from patients with other forms of cancer, i.e., renal cancer (13 samples), lung cancer (23 samples), and breast cancer (10 samples). The results are set forth in Table I which follow:

Clone Number	Normal Sera	Colon Cancer	Renal Cancer	Lung Cancer	Breast Cancer
NY-Co-8	0/16	8/29	1/13	0/23	0/10
NY-Co-9	0/16	5/29	1/13	1/23	0/10
NY-Co-13	0/16	5/29	0/13	0/23	0/10
NY-Co-16	0/16	3/29	0/13	0/23	0/10
NY-Co-20	0/16	4/29	0/13	0/23	0/10
NY-Co-38	0/16	4/29	3/13	0/23	1/10

Example 5

Following the screening work described supra, the cDNA inserts were purified and sequenced, following standard methods.

Of the six clones which were identified as being reactive with autologous and allogeneic cancer serum, and not with normal serum, two were found to be identical to previously identified molecules. Four others were found to have little or no homology to known sequences. These are presented as SEQ ID NOS: 1-4. Of twenty seven allogeneic colon cancer serum samples tested, 67% reacted with at least one of these antigens.

Example 6

The expression pattern of mRNA corresponding to SEQ ID NOS: 1, 2 and 4, as well as other sequences identified via the preceding examples was determined. To do this, RT-PCR was carried out on a panel of RNA samples, taken from normal tissue. The panel contained RNA of lung, testis, small intestine, colon, breast, liver and placenta tissues. The RNA was purchased from a commercial source. RNA from a colon tumor sample was also included. All samples were set up for duplicate runs, so that genomic DNA contamination could be accounted for. In the controls, no reverse transcriptase was used.

Primers were designed which were specific for the cDNA, which would amplify 5′-fragments, from 300-400 base pairs in length. The PCR reactions were undertaken at an annealing temperature of 68°C. Where appropriate, 5′ and 3′-RACE reactions were undertaken, using gene specific primers, and adapter primers, together with commercially available reagents. Specifically, SEQ ID NOS: 2 and 4 were tested using RACE. The

resulting products were subcloned into vector pCR 2.1, screening via PCR using internal primers, and then sequenced.

SEQ ID NOS: 1 and 2 were found to be amplified in all tissues tested. SEQ ID NO: 4 was found in colon tumor, colon metastasis, gastric cancer, renal cancer and colon cancer cell lines Colo 204 and HT29, as well as in normal colon, small intestine, brain, stomach, testis, pancreas, liver, lung, heart, fetal brain, mammary gland, bladder, adrenal gland tissues. It is was not found in normal uterine, skeletal muscle, peripheral blood lymphocytes, placental, spleen thymus, or esophagus tissue, nor in lung cancer.

The analysis also identified differential expression of a splice variant of SEQ ID NO: 4, i.e., SEQ ID NO: 5. When the two sequences were compared, it was found that SEQ ID NO: 4 encodes a putative protein of 652 amino acids, and molecular weight of 73,337 daltons. SEQ ID NO: 5, in contrast, lacks an internal 74 base pairs, corresponding to nucleotides 1307-1380 of SEQ ID NO: 4. The deletion results in formation of a stop codon at the splice function, and a putative protein of 404 amino acids, and molecular weight 45,839. The missing segment results in the putative protein lacking a PEST protein degradation sequence, thereby suggesting a longer half life for this protein.

In additional experiments, primers designed not to differentiate between SEQ ID NOS: 4 and 5 resulted in almost universal amplification (placenta being the only exception). In contrast, when primers specific for SEQ ID NO: 5 were used differences were seen in normal pancreatic, liver, lung, heart, fetal breain, mammary gland, bladder, and adrenal gland tissue, where there was no expression of SEQ ID NO: 5 found.

Example 7

Northern blotting was also carried out for SEQ ID NOS: 1, 2, 4 and 5. To do this, the same commercially available RNA libraries discussed supra were used.

Samples (2 ug) of polyA$^+$ RNA were analyzed from these samples, using random, ^{32}P labelled probes 300-360 nucleotides in length, obtained from PCR products. These probes were hybridized to the RNA, for 1.5 hours, at 68°C, followed by two washes at 0.1xSSC, 0.1% SDS, 68°C, for 30 minutes each time.

SEQ ID NOS: 1 and 2 were again found to be universally expressed.

Example 8

Further screening identified additional isoforms of SEQ ID NOS: 1 and 4. These are set forth as SEQ ID NOS: 6, 7 and 8. The isoform represented by SEQ ID NO: 6 is a naturally occurring splice variant of SEQ ID NO: 1, found in normal colon. SEQ ID NO: 7, which is an isoform of SEQ ID NO: 4, was found in brain tissue, primarily spinal chord and medulla. SEQ ID NO: 8, was found in normal kidney and in colon tumors, metastasized colon cancer, gastric cancer, and in colon cancer cell line Colo 205. It was not found in any normal tissue other than kidney.

The foregoing examples demonstrate several features of the invention. These include diagnostic methods for determining presence of transformed cells, such as colon cancer cells, in a sample. The sample may contain whole cells or it may be, e.g., a body fluid sample, or an effusion, etc., where the sample may contain cells, but generally will contain shed antigen. The experiments indicate that there is a family of proteins, expression of which is

associated with colon cancer. Hence, the invention involves, inter alia, detecting at least two of the proteins encoded by any of SEQ ID NOS: 1-5 wherein, presence of these is indicative of a pathology, such as colon cancer or other type of related condition. Exemplary of the type of diagnostic assays which can be carried out are immunoassays, amplification assays (e.g., PCR), or, what will be referred to herein as a "display array". "Display array" as used herein refers to a depiction of the protein profile of a given sample. Exemplary of such displays are 2-dimensional electrophoresis, banding patterns such as SDS-gels, and so forth. Thus, one aspect of the invention involves diagnosing colon cancer or a related condition by determining protein display of a sample, wherein a determination of at least one of the proteins, or expression of their genes, is indicative of colon cancer or a related condition. There are many ways to carry out these assays. For example, as indicated herein, antibodies to the proteins were found in patient samples. One can assay for these antibodies using, e.g., the methodology described herein, or by using a purified protein or proteins or antigenic fragment thereof, and so forth. One can also assay for the protein itself, using antibodies, which may be isolated from samples, or generated using the protein and standard techniques. This antibodies can then be labelled, if desired, and used in standard immunoassays. These antibodies or oligonucleotide probes/primers may also be used to examine biopsied tissue samples, e.g., to diagnose precancerous conditions, early stage cancers, and so forth.

Similarly, any and all nucleic acid hybridization systems can be used, including amplification assays, such as PCR, basic probe hybridization assays, and so forth. The antibodies, such as polyclonal antibodies, monoclonal antibodies, the hybridomas which

produce them, recombinantly produced antibodies, binding fragments of these, hybridization kits, DNA probes, and so forth, are all additional features of the invention.

Any of these assays can also be used in progression/regression studies. One can monitor the course of an abnormality such as colon cancer which involve expression of any one of the proteins, the expression of which is governed by the nucleic acid molecules SEQ ID NOS: 1-5, simply by monitoring levels of the protein, its expression, and so forth using any or all of the methods set forth supra.

As has been indicated supra, the isolated nucleic acid molecules which comprise the nucleotide sequences set forth in SEQ ID NOS: 1-5 are new, in that they have never been isolated before. These nucleic acid molecules may be used as a source to generate colon cancer specific proteins and peptides derived therefrom, and oligonucleotide probes which can themselves be used to detect expression of these genes. Hence, a further aspect of the invention is an isolated nucleic acid molecule which comprises any of the nucleotide sequences set forth in SEQ ID NOS: 1-5, or molecules whose complements hybridize to one or more of these nucleotide sequences, under stringent conditions, expression vectors comprising these molecules, operatively linked to promoters, cell lines and strains transformed or transfected with these, and so forth. "Stringent conditions", is used herein, refers to condition such as those specified in U.S. Patent No. 5,342,774, i.e., 18 hours of hybridization at 65°C, followed by four one hour washes at 2xSSC, 0.1% SDS, and a final wash at 0.2xSSC, more preferably 0.1xSSC, 0.1% SDS for 30 minutes, as well as alternate conditions which afford the same level of stringency, and more stringent conditions.

It should be clear that these methodologies may also be used to track the efficacy of a

therapeutic regime. Essentially, one can take a baseline value for the protein or proteins being tested, using any of the assays discussed supra, administer a given therapeutic, and then monitor levels of the protein or proteins thereafter, observing changes in protein levels as indicia of the efficacy of the regime.

The identification of the proteins and nucleic acid molecules set forth herein as being implicated in pathological conditions such as colon cancer also suggests a number of therapeutic approaches to such conditions. The experiments set forth supra establish that antibodies are produced in response to expression of these proteins, suggesting their use as a vaccine. Hence, a further embodiment of the invention is the treatment of conditions which are characterized by expression of one or more of the subject proteins, via immunotherapeutic approaches. One of these approaches is the administration of an amount of one or more these proteins, or an immunogenic peptide derived therefrom in an amount sufficient to provoke or augment an immune response. The proteins or peptides may be combined with one or more of the known immune adjuvants, such as saponins GM-CSF interleukins, and so forth. If the peptides are too small to generate a sufficient antibody response, they can be coupled to the well known conjugates used to stimulate responses.

Similarly, the immunotherapeutic approaches include administering an amount of inhibiting antibodies sufficient to inhibit the protein or proteins. These antibodies may be, e.g., antibodies produced via any of the standard approaches elaborated upon supra.

T cell responses may also be elicited by using peptides derived from the proteins which then complex, non-covalently, with MHC molecules, thereby stimulating proliferation of cytolytic T cells against any such complexes in the subject. It is to be noted that the T

cells may also be elicited in vitro, and then reperfused into the subject being treated.

Note that the generation of T cells and/or antibodies can also be accomplished by administering cells, preferably treated to be rendered non-proliferative, which present relevant T cell or B cell epitopes for response.

The therapeutic approaches may also include gene therapies, wherein an antisense molecule, preferably from 10 to 100 nucleotides in length, is administered to the subject either "neat" or in a carrier, such as a liposome, to facilitate incorporation into a cell, followed by inhibition of expression of the protein. Such antisense sequences may also be incorporated into appropriate vaccines, such as in viral vectors (e.g., Vaccinia), bacterial constructs, such as variants of the well known BCG vaccine, and so forth.

An additional DNA based therapeutic approach is the use of a vector which comprises one or more nucleotide sequences, preferably a plurality of these, each of which encodes an immunoreactive peptide derived from the expressed proteins. One can combine these peptides expressing sequences in all possible variations, such as one from each protein, several from one or more protein and one from each of the additional proteins, a plurality from some and none from others, and so forth.

Other features of the invention will be clear to the skilled artisan, and need not be repeated here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions

thereof, it being recognized that various modifications are possible within the scope of the invention.

We Claim:

1. An isolated nucleic acid molecule which comprises a nucleotide sequence, the complementary sequence of which hybridizes, under stringent conditions to at least one nucleic acid molecule having a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5.

2. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.

3. Cell line or cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 1.

4. Cell line or cell strain, transformed or transfected with the expression vector of claim 2.

5. The isolated nucleic acid molecule of claim 1, which consists of a nucleotide sequences set forth at SEQ ID NO: 1, 2, 3, 4 or 5.

6. Isolated protein encoded by the isolated nucleic acid molecule of claim 1.

7. Method for determining colon cancer in a sample, comprising assaying said sample for expression of the isolated nucleic acid molecule of claim 1, presence thereof being indicative of colon cancer.

8. Method for determining colon cancer in a sample, comprising assaying said sample for a protein encoded by the isolated nucleic acid molecule of claim 1, presence of said protein being indicative of colon cancer in said sample.

9. The method of claim 8, comprising assaying said sample for a peptide derived from said protein.

10. Composition of matter useful in treating colon cancer, comprising the isolated nucleic acid molecule of claim 1, and a pharmaceutically acceptable carrier.

11. Composition of matter useful in treating colon cancer, comprising therapeutically effective amount of the expression vector of claim 2, and a pharmaceutically acceptable carrier.

12. Composition of matter useful in treating colon cancer, comprising a therapeutically effective amount of the cell line or cell strain of claim 3, and having presented on its surface, a peptide complexed to an MHC molecule to form a complex which provokes an immune response against colon cancer cells.

13. A method for treating a subject afflicted with a colon cancer or related disorder, comprising:

(i) removing an immunoreactive cell containing sample from said subject,

(ii) contacting the immunoreactive cell containing sample to the cell line or cell strain of claim 3 conditions favoring production of cytolytic T cells against a peptide derived from the proteins encoded by the isolated nucleic acid molecule used to transfect or transform said cell, and

(iii) introducing said cytolytic T cells to said subject in an amount sufficient to lyse said cells.

14. A method for treating a subject afflicted with colon cancer or related condition, comprising:

(i) identifying a gene expressed by cancer cells associated with said colon cancer or related condition, wherein said gene comprises at least one nucleotide sequence set forth in SEQ ID NOS: 1-5;

(ii) identifying an MHC molecule which presents a portion of an expression product of said gene;

(iii) transfecting a host cell having the same molecule as identified in (ii) with said gene;

(iv) culturing said transfected cells to express said gene, and;

(v) introducing an amount of said cells to said subject sufficient to provoke an immune response against said cancer cells.

15. The method of claim 14, wherein said immune response comprises a B-cell response.

16. The method of claim 14, wherein said immune response comprises a T-cell response.

17. The method of claim 15, wherein said B cell response comprises production of antibodies specific to said expression product or peptide derived therefrom.

18. The method of claim 16, wherein said T-cell response comprises generation of cytolytic T-cell specific for cells presenting said peptide.

19. The method of claim 14, further comprising treating said cells to render them non-proliferative.

20. A method for treating a subject with a cancerous condition characterized by abnormal amounts of a protein, comprising:

(i) identifying a gene expressed by said abnormal cells, comprising at least one nucleotide sequence set forth in SEQ ID NOS: 1-5;

(ii) transfecting a host cell having the same MHC type as said patient expressing said gene;

(iii) culturing said transfected cells to express said gene, and;

(iv) introducing an amount of said cells to said subject sufficient to provoke an immune response against said cancerous condition.

21. The method of claim 20, further comprising treating said cells to render them non proliferative.

22. A method for treating a subject with a condition characterized by abnormal amounts of protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5, comprising administering to said subject an amount of a cell transfected with (i) a nucleic acid molecule which codes for said protein and (ii) a nucleic acid sequence which codes for an MHC molecule which presents a peptide derived from said protein wherein said peptide is presented by cells associated with said condition, sufficient to alleviate said condition.

23. Method of claim 22, further comprising treating said cell to render it non-proliferative.

24. A method for treating a subject afflicted with a condition characterized by an abnormal amount of protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, comprising administering to said subject an amount of a reagent consisting essentially of non-proliferative cells having expressed on its surface a peptide characteristic of said abnormal cells in an amount sufficient to elicit an immune response thereto.

25. A method for treating a subject afflicted with a condition characterized by an abnormal amount of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, comprising administering to said subject an antibody which specifically binds to said protein or a peptide derived therefrom, said antibody being coupled to a therapeutically useful agent, in an amount sufficient to treat said condition.

26. A method for treating a subject afflicted with a condition characterized by abnormal amounts of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, comprising administering to said subject a sample of non-proliferative cells which express said protein in an amount sufficient to alleviate said condition.

27. A method for preventing onset of a condition characterized by abnormal amounts of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, in a subject comprising administering an amount of a vaccine comprising said protein and an adjuvant in an amount sufficient to prevent onset of said cancerous condition in said subject.

28. A method for preventing onset of a condition characterized by abnormal amounts of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, in a subject comprising administering an amount of a vaccine

comprising a peptide derived from said protein in an amount sufficient to prevent onset of said condition in said subject.

29. A method for preventing onset of a condition characterized by abnormal amounts of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, in a subject comprising administering an amount of a vector which comprises a gene encoding said protein to a cell which is capable of expressing said protein or presenting a peptide derived therefrom, in an amount sufficient to prevent onset of said cancerous condition in said subject.

30. A method for treating a subject afflicted with a condition characterized by abnormal amounts of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, comprising:

(i) identifying cells from said subject which express abnormal amounts of said protein;

(ii) isolating a sample of said cells;

(iii) cultivating said cell, and

(iv) introducing said cells to said subject in an amount sufficient to provoke an immune response against said cells.

31. The method of claim 30, further comprising rendering said cells non proliferative, prior to introducing them to said subject.

32. A method for treating a subject with a condition characterized by abnormal amounts of at least one protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth in any of SEQ ID NO: 1, 2, 3, 4 or 5, comprising administering to said subject a vector which comprises a sequence encoding at least one immunoreactive peptide derived from said at least one protein, to a cell capable of expressing and presenting said at least one peptide, in an amount sufficient to induce an immune response in said subject.

33. A method for following progress of a therapeutic regime designed to alleviate a condition characterized by abnormal expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5, comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) a peptide derived from said protein, (ii) a cytolytic T cell specific for cells presenting said peptide, and (iii) an antibody which specifically binds to said peptide of said protein, at a first time period;

(b) assaying level of the parameter selected in (a) at a second period of time and comprising it to the level determined in (a) as a determination of effect of said therapeutic regime.

34. A method for treating a pathological cell condition characterized by aberrant expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth at SEQ ID NO: 1, 2, 3, 4 or 5 associated with said condition, comprising

administering to a subject in need thereof an effective amount of either: (a) a protein inhibitor, or (b) an inhibitor of gene expression of said protein.

35. The method of claim 34, wherein said protein inhibitor is an inhibiting antibody.

36. The method of claim 34, wherein said inhibitor of gene expression is an antisense molecule.

37. A composition of matter useful in stimulating an immune response to a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5, comprising a plurality of peptides derived from the amino acid sequence of said protein, wherein said peptides bind to one or more MHC molecules presented on the surface of cells which express an abnormal amount of said protein.

38. The composition of matter of claim 37, wherein at least a portion of said plurality of peptides bind to MHC molecules and elicit a cytolytic response thereto.

39. The composition of matter of claim 38, further comprising an adjuvant.

40. The composition of matter of claim 39, wherein said adjuvant is a saponin, GM-CSF, or an interleukin.

41. An isolated antibody which binds to a protein encoded by a nucleic acid molecule which comprises the nucleotide sequence of any of SEQ ID NO: 1, 2, 3, 4 or 5.

42. The isolated antibody of claim 41, wherein said antibody is a monoclonal antibody.

43. An isolated antibody which specifically binds to a peptide derived from a protein encoded by a nucleic acid molecule which comprises a nucleotide sequence set forth in any of SEQ ID NO: 1, 2, 3, 4 or 5.

44. The antibody of claim 43, wherein said antibody is a monoclonal antibody.

45. An isolated antibody which specifically binds to a complex of (i) a peptide derived from protein encoded by a nucleic acid molecule which comprises a nucleotide sequence set forth in any of SEQ ID NO: 1, 2, 3, 4 or 5 and (ii) an MHC molecule to which said peptide complexes, but does not bind to (i) or (ii) alone.

46. The antibody of claim 45, wherein said antibody is a monoclonal antibody.

47. Method for determining regression, progression or onset of a condition characterized by abnormal levels of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5, comprising monitoring a

sample from a patient with said condition for a parameter selected from the group consisting of (i) protein, (ii) a peptide derived from said protein and (iii) cytolytic T cells specific for a peptide derived from said protein and an MHC molecule to which is bound is indicative of progression or regression or onset of said condition.

48. The method of claim 47, wherein said sample is a body fluid or effusion.

49. The method of claim 47, wherein said sample is a tissue.

50. The method of claim 47, wherein contacting said sample with an antibody which specifically binds with said protein or peptide.

51. The method of claim 50, wherein said antibody is labelled with a radioactive label or an enzyme.

52. The method of claim 50, wherein said antibody is a monoclonal antibody.

53. The method of claim 47, comprising amplifying RNA which codes for said protein.

54. The method of claim 53, wherein said amplifying comprises carrying out polymerase chain reaction.

55. The method of claim 47, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said SCP protein.

56. The method of claim 47, comprising assaying said sample for said peptide.

Abstract Of The Disclosure

Various molecules associated with disorders such as colon cancer are disclosed. The invention also discloses diagnostic and therapeutic methods based upon these molecules.

LUD 5506-JEL/NDH

Express Mail Label No:
EL024661754US
Date of Deposit: **2-11-00**

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My resident, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ISOLATED NUCLEIC ACID MOLECULES ASSOCIATED WITH COLON CANCER AND METHODS FOR DIAGNOSING AND TREATING COLON CANCER, the specification of which

( ) is attached hereto.
(X) was filed on October 10, 1997 as Application Serial No. 08/948,705
and was amended on (1) _____, (2) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, 1.56(a).

Foreign Priority Applications
I hereby claim foreign priority benefits under Title 35, United States Code 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

			Priority Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	Yes ( ) No ( )
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	Yes ( ) No ( )

U.S. Priority Applications
I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

_____ (Applic. Serial No.)	_____ (Filing Date)	_____ (Status-patented/pending/abandoned)
_____ (Applic. Serial No.)	_____ (Filing Date)	_____ (Status-patented/pending/abandoned)

LUD 5506-JEL/NDH

Power of Attorney

I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: John E. Lynch, Reg. No. 20,940; Peter F. Felfe, Reg. No. 20,297; Norman D. Hanson, Reg. No. 30,946; Andrew L. Tiajoloff, Reg. No. 31,575; John A. Bauer, Reg. No. 32,554; Mary Anne Schofield, Reg. No. 36,669; Madeline F. Baer, Reg No. 36,437 and James R. Crawford, Reg. No. 39,155, my attorneys with full power of substitution and revocation. Address all telephone calls to Norman D. Hanson, at (212) 688-9200. Address all correspondence to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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LUD 5506-JEL/NDH

DECLARATION FOR PATENT APPLICATION

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( ) is attached hereto.
(X) was filed on October 10, 1997 as Application Serial No. 08/948,705 and was amended on (1) _____, (2) _____ (if applicable).

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			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	Yes ( ) No ( )
(Number)	(Country)	(Day/Month/Year Filed)	Yes ( ) No ( )

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(Applic. Serial No.)	(Filing Date)	(Status-patented/pending/abandoned)
(Applic. Serial No.)	(Filing Date)	(Status-patented/pending/abandoned)

Power of Attorney

I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: John E. Lynch, Reg. No. 20,940; Peter F. Felfe, Reg. No. 20,297; Norman D. Hanson, Reg. No. 30,946; Andrew L. Tiajoloff, Reg. No. 31,575; John A. Bauer, Reg. No. 32,554; Mary Anne Schofield, Reg. No. 36,669; Madeline F. Baer, Reg. No. 36,437 and James R. Crawford, Reg. No. 39,155, my attorneys with full power of substitution and revocation. Address all telephone calls to Norman D. Hanson, at (212) 688-9200. Address all correspondence to:

FELFE & LYNCH
805 Third Avenue
New York, New York 10022

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date of Deposit: **2-11-00**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Scanlan et al.
Serial No.:	08/948,705
Filed:	October 10, 1997
For:	ISOLATED NUCLEIC ACID MOLECULES ASSOCIATED WITH COLON CANCER AND METHODS FOR DIAGNOSING AND TREATING COLON CANCER
Examiner:	J.S. Brusca
Art Unit:	1636

Assistant Commissioner for Patents
Washington, D.C. 20231

ASSOCIATE POWER OF ATTORNEY

Sir:

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Stanley Sacks	19,900	Gary S. Engelson	35,128	Michael G. Verga	39,410
Edward F. Perlman	28,105	Peter J. Gordon	35,164	Robert E. Rigby, Jr.	36,904
Lawrence M. Green	29,384	Randy J. Pritzker	35,986	Robert A. Skrivanek,Jr.	41,316
Steven J. Henry	27,900	Richard F. Giunta	36,149	Robert M. Abrahamsen	40,886
Therese A. Hendricks	30,389	Douglas R. Wolf	36,971	Lesley A. Hamlin	41,054
Edward R. Gates	31,616	Elizabeth R. Plumer	36,637	Lindsay G. McGuinness	38,549
William R. McClellan	29,409	Timothy J. Oyer	36,628	Mike W. Crosby	40,970
Ronald J. Kransdorf	20,004	John N. Anastasi	37,765	Ivan D. Zitkovsky	37,482
M. Lawrence Oliverio	30,915	Helen C. Kindregan	39,248	Michele J. Young	P-43,299
James J. Foster	30,052	James M. Hanifin,Jr.	39,313	Edward J. Russavage	P-43,069
Charles E. Pfund	17,030	Christopher S. Schultz	37,929	Alan B. Sherr	P-42,147
Jason M. Honeyman	31,624	Paul D. Sorkin	39,039		

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Federal Reserve Plaza
600 Atlantic Avenue
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Respectfully submitted,

FULBRIGHT & JAWORSKI, L.L.P.

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SEQUENCE LISTING

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